Remarks and Arguments

Amendments to the Claims

Claims 1-39 were pending in this application. Claims 3, 6, 20, 21 and 23-39 were withdrawn by the Examiner as being drawn either to non-elected subject matter or to non-elected species of the invention. All the claims currently under examination stand rejected.

Claim 1 has been amended to recite a mouse β-actin promoter. Support for this amendment is found, e.g., in paragraph [0119] of Example 1 of the present application, published as US Patent Application Publication No. 2008/0250514. Claim 1 has also been amended to specify that the enhancer is a Cytomegalovirus (CMV) enhancer. Support for this amendment is found in newly canceled claim 2. Claims 5 and 8 have been amended to preserve proper antecedent basis.

Claim 14 has been amended to recite that the cell comprises an activated oncogene. Claim 15 has been amended to recite that the activated oncogene is on a vector in the cell. Support for these amendments is found, e.g., in paragraphs [0082] and [0083] of the present application.

Claims 22 and 23 have been amended for clarity.

New claims 40-45 have been added. Applicants believe these claims belong in the presently elected restriction group, and so should be examined with the present group. The new claims are supported, e.g., by the disclosure at Fig. 1 and paragraphs [0014]-[0016] and [0119]-[0147] of the application, which describe the cloning of the mouse β–actin promoter (SEQ ID NO:2) away from mouse β–actin genomic sequence, the manipulation of that promoter sequence, and the insertion of that promoter sequence into a vector upstream of a DNA encoding luciferase. See also paragraphs [0065] and [0074]-[0077]. New claims 41, 42, 44 and 45 are further supported, e.g., by the disclosure at Fig. 1 and in paragraph [0191] of the application, which describes cloning of the CMV enhancer comprising the nucleotide sequence shown in SEQ ID NO: 4 into a vector, which vector comprises the mouse β–actin promoter comprising the nucleotide sequence shown in SEQ ID NO:2.

Claims 2, 4, 19 and 20 have been canceled without prejudice.

No new matter has been added as a result of the present amendments. Applicants reserve the right to pursue any subject matter canceled as a result of the present amendments in future prosecution, either in this application or in one or more continuing applications.

After entry of the present claim amendments, claims 1, 5, 7-18, 22, and 40-45 will be pending and under consideration in this application. Once one or more of those claims is deemed allowable, Applicants request rejoinder (as appropriate) and examination of restricted-out method claims (e.g., claims 23-29, 38, and 39).

Objection to the Specification

The specification was objected to for including embedded hyperlinks and/or other form of browser-executable code. Paragraphs [0119] and [0199] of the published application have been amended to remove the embedded hyperlinks. Applicants submit that the specification as presently amended complies with the provisions of 37 CFR 1.57(d), and request that this objection be withdrawn.

Rejections under 35 U.S.C. §112, Second Paragraph

Claims 14-16 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for reciting "the oncogene" and "the transactivator" without sufficient antecedent basis. The amendments to claims 14 and 15 render this rejection moot, and Applicants request that it be withdrawn.

Rejections under 35 U.S.C. §103

Hadjantonakis et al in view of Estes et al.

Claims 1, 2, 4, 7, 8, 12, 13, 17-19, and 22 were rejected under 35 U.S.C. §103 as being obvious over Hadjantonakis *et al.* (Mech. Develop. 76:79-90, 1998) in view of Estes *et al.* (U.S. Patent No. 7,423,135). Hadjantonakis *et al.* is cited for disclosing a vector for expressing a transgene in mice, the vector including a CMV immediate early enhancer and a chicken β–actin promoter. Estes *et al.* is cited for disclosing a mouse β–actin promoter. The Examiner asserts that it would have been obvious to combine the teachings of Hadjantonakis *et al.* and Estes *et al.*

to arrive at the presently claimed DNA constructs, vectors and cells recited in claims 1, 2, 4, 7, 8, 12, 13, 17-19, and 22. Without conceding the merits of this rejection, Applicants have canceled claims 2, 4 and 19 without prejudice, and have amended claim 1. Applicants traverse this rejection as it may be applied to currently amended claim 1 and to claims 7, 8, 12, 13, 17, 18, and 22, each of which depends from and thereby incorporates the features recited in claim 1, and as it may be applied to newly added claims 40-45.

The present application discloses that a DNA construct comprising a mouse β–actin promoter and a CMV enhancer, as recited in amended independent claim 1, can drive an unexpectedly high level of expression. For example, Example 2 of the present application shows that the mouse β–actin promoter exhibits a significantly higher level of activity than the human EF1α promoter (the "CEF promoter"). These results are shown graphically in Figure 2. In Figure 2, the bar labeled "pmAct-Luc-neo" shows the activity of a luciferase reporter gene driven by the mouse β–actin promoter, while the bar labeled "pCEF-Luc-neo" shows the activity of a luciferase reporter gene driven by the CEF promoter. As can be seen, the mouse β–actin promoter drives a significantly higher level of luciferase reporter activity than the CEF promoter.

Moreover, Example 2 of the present application demonstrates that the activity of the mouse β–actin promoter is significantly increased when combined with a CMV enhancer. These results are shown graphically in Figure 3. In Figure 3, the bar labeled "pmAct-Luc-neo" shows the activity of a luciferase reporter gene driven by the mouse β–actin promoter alone, while the bar labeled "phCMV-mAct-Luc-neo" shows the activity of a luciferase reporter gene driven by the mouse β–actin promoter operably linked to a CMV enhancer. As can be seen, the combination of the mouse β–actin promoter and the CMV enhancer results in a greatly increased level of luciferase reporter activity as compared to the mouse β–actin promoter alone.

In further support of the non-obviousness of the presently pending claims, Applicants submit with this reply a declaration under 37 CFR §1.132 signed by Mr. Hiroyuki Tsunoda, one of the two named inventors on the present application. The declaration describes an experiment comparing (1) expression of a luciferase reporter gene driven by the presently claimed combination of a mouse β–actin promoter and a CMV enhancer, with (2) expression of a luciferase reporter gene driven by the chicken β–actin promoter in combination with a CMV enhancer. This experiment was conducted in accordance with the experimental procedures

described in Example 2 of the present application. The results are shown in Exhibit B attached to the declaration. As can be seen in the Exhibit B histogram, a vector comprising the mouse β–actin promoter and the human CMV enhancer ("CMV-mAct") drove significantly higher levels of luciferase expression than a conventional vector comprising the chicken β–actin promoter and the human CMV enhancer ("CAG"). Indeed, even in the absence of CMV enhancer, a vector comprising the mouse β–actin promoter ("mAct") drove a higher level of luciferase expression than did the vector comprising both the chicken β–actin promoter and the human CMV enhancer ("CAG"). These unexpected properties of the mouse β–actin promoter, and particularly the mouse β–actin promoter/human CMV enhancer combination, are neither taught nor suggested by Hadjantonakis *et al.* or Estes *et al.*

The Examiner states that "A person of ordinary skill in the art would construct such an expression construct [a mouse β–actin promoter in combination with a CMV enhancer] in an expression vector as a matter of design choice, which amounts to simple substitution of one known element for another to obtain predictable results." In light of the discussion above, Applicants submit that the results in the present situation are not "predictable results." The present inventors have shown that a DNA construct comprising mouse β–actin promoter and a CMV enhancer, as recited in claim 1, exhibits an unexpected level of promoter activity, which level is surprisingly higher than would have been expected based on the cited art. Neither Hadjantonakis *et al.* nor Estes *et al.* suggests that a DNA construct comprising a mouse β–actin promoter and a CMV enhancer can drive such a high level of reporter gene expression.

New independent claims 40 and 43 are distinguished over the prior art for different reasons. Applicants point out that the sequence of the mouse β–actin promoter disclosed in Estes *et al.* differs from the sequence of the mouse β–actin promoter specified in claims 40 and 43 in at least two ways.

First, as acknowledged in the Office action at page 6, the mouse β–actin promoter of the present application (identified in the present application as SEQ ID NO: 2) lacks the 5'-end 1412 nucleotides of the 2953 nt[1] mouse β–actin promoter of Estes *et al.* (identified in Estes *et al.* as SEQ ID NO: 3). The generic list of possible fragments of SEQ ID NO: 3 in Estes *et al.* at

[1] The Office action at page 6 says "2593", but Estes *et al.*'s SEQ ID NO: 3 is 2953 nt in length.

column 5, lines 61-66, gives no experimental evidence of activity. In fact, Estes *et al.* did not even bother to test their full-length (2953 nt) mouse β–actin promoter for promoter activity, so it cannot be determined from the disclosure of Estes *et al.* whether even the full-length sequence disclosed therein is functional. (See Example 6 of Estes *et al.*, which merely sketches out how the test could be conducted and opines that the promoter is "expected" to work.) No other prior art cited in the Office action provides a suggestion that one could omit nearly half of the 2953 nt sequence disclosed in Estes *et al.* and end up with a functional promoter, much less one that functions as powerfully as the presently claimed 1542 nt SEQ ID NO: 2.

Second, the sequence of the presently claimed SEQ ID NO: 2 at positions 305-318 differs from the corresponding sequence (positions 1717-1730) of Estes *et al.*'s SEQ ID NO: 3, in that the present claimed SEQ ID NO: 2 contains an additional thymidine residue as compared to the Estes *et al.* sequence. See discussion in paragraph [0133] in Example 1 of the present application. Nothing in Estes *et al.* nor the other cited art would have suggested incorporating an extra thymidine residue at that position.

For at least these reasons, new claims 40 and 43 are neither anticipated nor obvious in view of the cited art. Each of newly added claims 41-42 depends from and thereby incorporates the features recited in claim 40, and each of newly added claims 44-45 depends from and thereby incorporates the features recited in claim 43, so the same arguments apply to them.

Thus, neither Hadjantonakis *et al.* nor Estes *et al.*, either alone or in combination, renders obvious the pending claims as currently amended. Applicants request that this rejection be withdrawn.

Estes et al. in view of Debs et al.

Claims 1, 2, and 5 were rejected under 35 U.S.C. §103 as being obvious over Estes *et al.* in view of Debs *et al.* (U.S. Patent No. 6,468,798). As acknowledged by the Examiner, Estes *et al.* do not disclose that the nucleotide sequence of the CMV enhancer is the sequence identified as SEQ ID NO: 4 in the present application. The Examiner asserts, however, that Debs *et al.* cures the deficiency of Estes *et al.* Claim 2 is canceled, so the rejection is moot as to that claim. Applicants traverse this rejection as it may be applied to currently amended claims 1 and 5.

As discussed above, Estes *et al.* fails to teach or suggest that a DNA construct comprising a mouse β–actin promoter and a CMV enhancer can drive reporter gene expression to the surprisingly high level described above. Nothing in Debs *et al* gives one of ordinary skill in the art a reason to expect the significant improvement in activity that Applicants achieved with the combination of claim 1. The results remain surprising in view of the art. Thus, Debs *et al.* fails to cure the deficiency of Estes *et al.* with respect to claim 1 and its dependent claim 5.

In addition to the above arguments, Applicants have established above that the mouse β-actin promoter sequence disclosed in Estes *et al.* does not comprise the sequence of SEQ ID NO: 2 (as required by claim 5), as the Estes et al. sequence lacks a thymidine residue found within SEQ ID NO: 2. Debs *et al.* does not correct this deficiency of Estes et al. Thus, the cited art does not meet the limitation of claim 5 regarding SEQ ID NO: 2.

Applicants also point out that Debs *et al.* does not make up for the above-described deficiencies of Estes *et al.* with respect to newly added claims 40-45.

It is clear that neither Estes *et al.* nor Debs *et al.*, either alone or in combination, renders obvious the pending claims as currently amended. Applicants request that this rejection be withdrawn.

Estes et al. in view of Yano et al.

Claims 1, 7-11 and 14-16 were rejected under 35 U.S.C. §103 as being obvious over Estes *et al.* in view of Yano *et al.* (Cytotech. 16:167-178, 1994). As acknowledged by the Examiner, Estes *et al.* do not disclose inclusion of a c-Ha-ras oncogene in vectors comprising a β–actin promoter and a CMV enhancer. The Examiner asserts, however, that Yano *et al.* cures the deficiency of Estes *et al.* by disclosing that the c-Ha-ras oncogene can enhance promoter activity when expressed downstream of a CMV promoter-driven construct. Applicants traverse this rejection as it may be applied to the amended claims and to new claims 40-45.

As discussed above, Estes *et al.* provides no reason to expect that a DNA construct comprising a mouse β–actin promoter and a CMV enhancer can drive reporter gene expression to the surprising level demonstrated by the present application. Yano *et al.* is cited for its disclosure of the use of the c-Ha-ras oncogene, in order to meet the limitations of certain dependent claims. Nowhere, however, does Yano *et al.* provide what is missing from Estes *et al.*

with respect to claim 1 and its dependents, i.e., a reason to expect that a DNA construct comprising a mouse β–actin promoter and a CMV enhancer can drive reporter gene expression to a degree greater than observed with the closest prior art. Further, Yano *et al.* does not make up for the deficiencies of Estes *et al.* with respect to newly added claims 40-45.

Thus, neither Estes *et al.* nor Yano *et al.*, either alone or in combination, renders obvious the pending claims as currently amended. Applicants request that this rejection be withdrawn.

Conclusion

In light of the present amendments and remarks, Applicants submit that the application is in condition for allowance, and respectfully request a notice to that effect. If the Examiner feels that a phone call would further prosecution or expedite allowance, the undersigned can be reached at (612) 335-5070.

Please apply the extension of time fee and any other required charges, or credit any overpayments, to deposit account 06-1050, referencing Attorney's Docket Number 14875-0162US1.

Respectfully submitted,

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